# AUTOMATED APPROACHES TO PROCESS DEVELOPMENT AND MANUFACTURE OF HUMAN T-CELLS AND MESENCHYMAL STEM CELLS USING SINGLE-USE BIOREACTOR TECHNOLOGIES

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Key Words: Cell and Gene Therapy, hMSCs, T-cell Bioprocessing, Automation, Single-Use Technologies

Improvements to process development and manufacturing technology will have a significant impact in reducing the overall costs associated with the manufacture and scale-up of cell and gene therapies. Small-scale models, including single-use technologies such as microbioreactors, play a critical role in this regard as they reduce reagent requirements and can facilitate high-throughput screening of process parameters and culture conditions.

This talk will demonstrate the amenability of the automated, single-use systems such as the ambr15® and ambr250® platforms for adherent human mesenchymal stem cell (hMSC) culture and suspension T-cell culture. We have also demonstrated that such systems can be used for effective bioprocess development of both a microcarrier and suspension cell line process, with data being validated in larger-scale studies.

For the hMSC studies, adhere culture on microcarriers was achieved through a combination of strategies including adapting the free suspension design of the vessel to improve the suspension and mixing of the microcarriers. A more effective cell attachment method was also developed by using only 50% of the final working volume of medium for the first 24 h combined with an intermittent agitation strategy. These improvements led to a reduction in the initial lag phase which in turn resulted in > 150 % increase in viable cell density after 24 h compared to the original process (no agitation for 24 h and 100 % working volume). Using the same methodology as in the ambr15®, similar improvements were obtained in larger scale spinner flask studies.

This improved bioprocess methodology, which was developed for a serum-based medium process, was applied to a serum-free process in the ambr15; this resulted in > 250% increase in yield compared to the ambr15 serum-based process. The use of the ambr15, with its improved control compared to the spinner flask, reduced the coefficient of variation on viable cell density in the serum containing medium from 7.65% to 4.08%, and the switch to the serum free medium further reduced these to 1.06% and 0.54% respectively. The combination of both serum-free and automated processing improved the consistency more than 10-fold compared to the initial manual, serum-based spinner flask work.

Similar work has been undertaken with the ambr250®, where a new single-use bioreactor vessel was designed to improve adherent microcarrier culture and has demonstrated improved growth of suspension T-cells for immunotherapy applications.

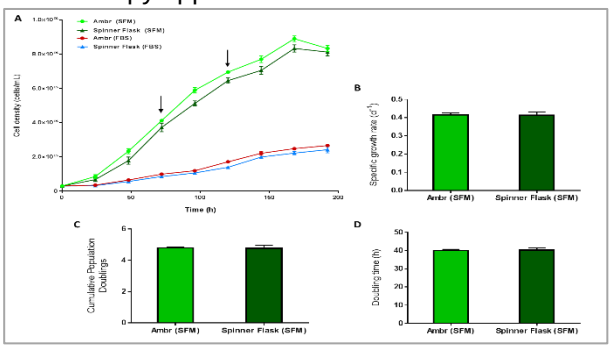


*Figure 1 – Growth kinetics of hMSCs for serum-free (SFM) and fetal bovine serum (FBS)-based media in both the ambr15 and spinner flasks with data showing (A) the viable cell density, (B) specific growth rate, (C) the cumulative population doublings and (D) the doubling time. Data show mean ± SD, n = 8.*

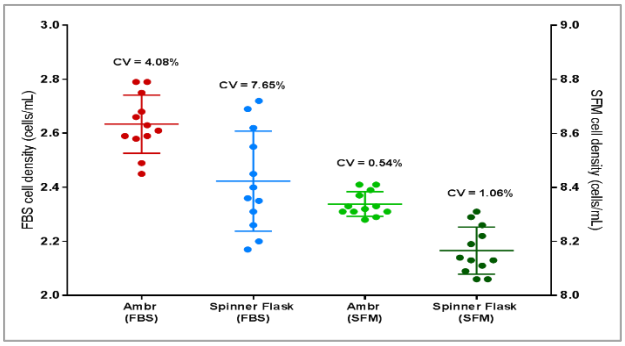


*Figure 2 – Extent of viable cell density variation in the ambr15 and spinner flask for both serum-free (SFM) and fetal bovine serum (FBS)-based cultures. Cell density values for FBS are aligned with the left y-axis and the SFM values with the right y-axis. Data show coefficient of variation (CV), n = 8.*